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Differetiation of *Adonis* L. in Japan VI. Foliar Characters and Pedicels

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Morphological characters of leaf and pedicel were examined in diploid, triploid, and tetraploid plants of *Adonis amurensis* Regel & Radde in Japan. The characters examined are not helpful enough to distingish ploidy level, except for the structure in pith cavity of pedicels at the fruiting season. It suggests that the results by previous authors need to be reexamined.

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Key words: *Adonis*, foliar characters, morphology, Ranunculaceae

In a previous paper I studied (1) the morphological variation of sepals and petals in the process of floral development and the difference between diploids and tetraploids, (2) in the shape of aggregate fruits, in the number of achenes per aggregate fruit and (3) in seed coat patterns and color. And I reported that the diploids and tetraploids were distinguished clearly by the characters of aggregate fruits and seeds (Suda 1998).

In the present study further questions of stomatal size, foliar morphology, and anatomical characters are addressed in relation to ploidy level.

Materials and Methods

Materials were collected in Iwate Prefecture, northeastern Honshu, Japan (Table 1). Fourteen plants from four diploid populations, seven triploid plants from three sites, and 20 plants from seven tetraploid populations were provided for the measurement of stomata. The sampling of fully matured leaves, which developed at the base of pedicels of first flowers, was made in the field at random just before the mature achenes were detached from aggregate fruits. Only one leaf from each individual was collected. Leaves from tetraploid populations and triploid plants were collected in the first week of May, and those from the diploid ones were collected in the beginning of June. The collected leaves were fixed in FAA solution in the field and stored in a deep freezer at – 20 °C.

The epidermis was cut off from the abaxial side of four secondary leaflets of the bipinnate leaf. Four temporary slides per individual were made and the lengths of 25 stomata from each slide (100 in total) were measured at random under the microscope at the magnification of × 400.

To compare foliar shapes, leaves that developed at the base of pedicels of first flowers were collected at the end of the fruiting season from six diploid and five tetraploid populations and 13 triploid individuals. They were pressed and dried for herbarium specimens. The specimens were xeroxed and the copies were used for the measurements.

In the case of bipinnate leaves of *Adonis*, the leaf length/leaf width value was not adequate as a foliar character because the angle of primary leaflets to rachis varied much in the process of completing herbarium specimens, making the leaf width various. Therefore, I used the ratio of leaf length to the first primary leaflet length (longer of the two) instead of the leaf length/leaf width value.

Pedicels were sampled twice: at the time (1) when the flowers were in full bloom and (2) when the mature achenes were detached from aggregate fruits. They were fixed in FAA solution in the field and stored in a deep freezer at – 20 ℃. Cross-sections were made by the usual paraffin method and stained with safranin-fast green staining method. The direction of microtome-sectioning was from the top, a few mm below the flower or the aggregate fruit, to the base.

The number of vascular bundles, the presence of reticular structure in the pith cavity, and of sclerenchyma cells on the peripherary zone of vascular bundles at the peak of blooming and at the end of fruiting season were examined under the microscope.

Preliminary observations showed that these anatomical characters were similar not only among individuals which belonged to the same population but also among populations which had the same chromosome numbers. Therefore, I attempted a comparison among diploid, triploid, and tetraploid plants as a whole. 42 diploid, 19 triploid and 44 tetraploid plants were provided in total and more than one pedicel per individual were sampled (Table 1).

Results

(1) *Stomatal Size*

Mean lengths and the standard deviations obtained from 41 individuals of 12 populations are summarized in Fig. 1. It demonstrated a tendency to increase the size in parallel with polyploid variation. But, the size varied so continuously between the minimum value in diploids and the maximum one in tetraploids that no break was recognized among diploid, triploid, and tetraploid level. The results suggested that the stomatal size was little use to distinguish the ploidy level.

(2) *Leaf Shapes*

By comparing the length of the second primary leaflet with that of the first primary one, leaves were classified into three types (Fig. 2). Type A consisted of leaves in which the first primary leaflets were longer than the second primary ones, while in type B the first and the second primary leaflets were similar in length. Leaves belonging to C type had second leaflets which were longer than the first primary ones (Fig. 2). The most common leaf shape was type A. The length and the length/leaflet length of leaves belonging to type A were measured and are summarized in Fig. 3.

Leaf length From the preliminary examination, it was confirmed that the leaf length varied little within a single individual but varied more among individuals within the same population. Therefore, I made a comparison of the leaf length among populations. The results showed that mean values were different among populations but, taking the standard deviation into consideration, there was little difference among populations and among diploid, triploid, and tetraploid level as well (Fig. 3).

Leaf length/leaflet length The values obtained for each population were also similar with each other as in the case of leaf lengths (Fig. 3). No clear difference was recognized among diploid, triploid, and tetraploid level either.

Hairiness Hairiness of the leaves and pedicels was examined for 15 individuals which were selected at random from diploid and tetraploid populations. 15 triploid plants were also examined. The results are summarized in Table 2. Although hair density varied among diploid, triploid, and tetraploid

Table 1. List of localities of populations, abbreviations of each population, numbers of materials examined and voucher specimens of *Adonis amurensis*

Locality*, abbreviation of each population and voucher		Number of plants examined**			
		Stm	Lf	Pd	
				Bl	Fr
<Diploid Populations>					
Toriyasawa, Tamayama-mura 玉山村，鳥谷沢 Suda TY52277 (IUM)	TY52277	2			2 (4)
Nakakugi-no-taira, Tamayama-mura 玉山村，中釘ノ平 Suda NK241577 (IUM)	NK241577	2	44		2 (8)
Shirasawa, Tamayama-mura 玉山村，白沢 Suda SS151577 (IUM)	SS151577	4		5 (12)	4 (10)
Takakubo, Tamayama-mura 玉山村，高久保 Suda TK33073 (IUM)	TK33073	6			7 (23)
Kamosori, Tamayama-mura 玉山村，鴨反 Suda KH5884 (IUM)	KH5884		35		
Nakatsuka, Tamayama-mura 玉山村，中塚 Suda NT5884 (IUM)	NT5884		33		
Mukaikudo, Iwate-machi 岩手町，向久保 Suda MK5884 (IUM)	MK5884		37	6 (10)	
Kamimura, Nishine-chô 西根町，上村 Suda KM5884 (IUM)	KM5884		50	11 (13)	
Shimoyamakozawa, Nishine-chô 西根町，下山子沢 Suda SY52785 (IUM)	SY52785		22	5 (12)	
<Populations having Triploid Plants>					
Toriyasawa, Tamayama-mura 玉山村，鳥谷沢	TY52277	4			4 (16)
Shirasawa, Tamayama-mura 玉山村，白沢	SS152278	2	1		3 (11)
Takakubo, Tamayama-mura 玉山村，高久保	TK33073	1			1 (6)
Tobita, Tamayama-mura 玉山村，飛田	TT5386		1	1 (6)	
Akasaka, Tamayama-mura 玉山村，赤坂	AS51584		3	3 (16)	
Nakakugi-no-taira, Tamayama-mura 玉山村，中釘ノ平	NK141577		3	1 (5)	
Same 同上	NK51191			1 (5)	
Makibori, Tamayama-mura 玉山村，巻堀	MH43086		1		
cv. Fukujukai 福寿海（栽培）	CVFJK		4	5 (26)	
<Tetraploid Populations>					
Fukizawa, Daitô-chô 大東町，吹沢 Suda FZ4374 (IUM)	FZ4374				2 (3)
Kami-ôkago, Fujisawa-chô 藤沢町，上大篭 Suda TG32173 (IUM)	TG32173	3			3 (5)
Aozasa, Tôno-city 遠野市，青笹	AM32473	2			2 (6)
Kudashizawa, Hanamaki-city 花巻市，下シ沢 Suda MD42274 (IUM)	MD42274	2			2 (6)
Tashiro, Kawai-mura 川井村，田代 Suda TS4973 (IUM)	TS4973	5		5 (11)	8 (9)
Gomyôjin, Sizukuishi-chô 雫石町，御明神 Suda AZ41173 (IUM)	AZ41173	3			2 (5)
Takakô, Tamayama-mura 玉山村，鷹高 Suda TT33073 (IUM)	TT33073	3			3 (8)
Furuyashiki, Tamayama-mura 玉山村，古屋敷 Suda FH42384 (IUM)	FH42384		48		
Hinoto, Tamayama-mura 玉山村，日ノ戸 Suda HT41380 (IUM)	HT41380		38	5 (10)	
Hirukubo, Tamayama-mura 玉山村，昼久保 Suda HK52481 (IUM)	HK52481		39	5 (9)	
Ashidauchi, Iwate-machi 岩手町，芦田内 Suda AU42384 (IUM)	AU42384		40		
Ômori, Iwate-machi 岩手町，大森 Suda OM52584 (IUM)	OM52584		38	5 (9)	
Chayaba, Kuzumaki-chô 葛巻町，茶屋場 Suda CY4673 (IUM)	CY4673	2			2 (6)

* All localities are in Iwate Prefecture.

** Stm: number of individuals used to examine the size of stomata. Lf: number of individuals used to examine the shape of leaves. Pd: number of individuals and pedicels (in parentheses) used to examine anatomical features. Bl: collected at the peak of blooming. Fr: collected at the end of fruiting.

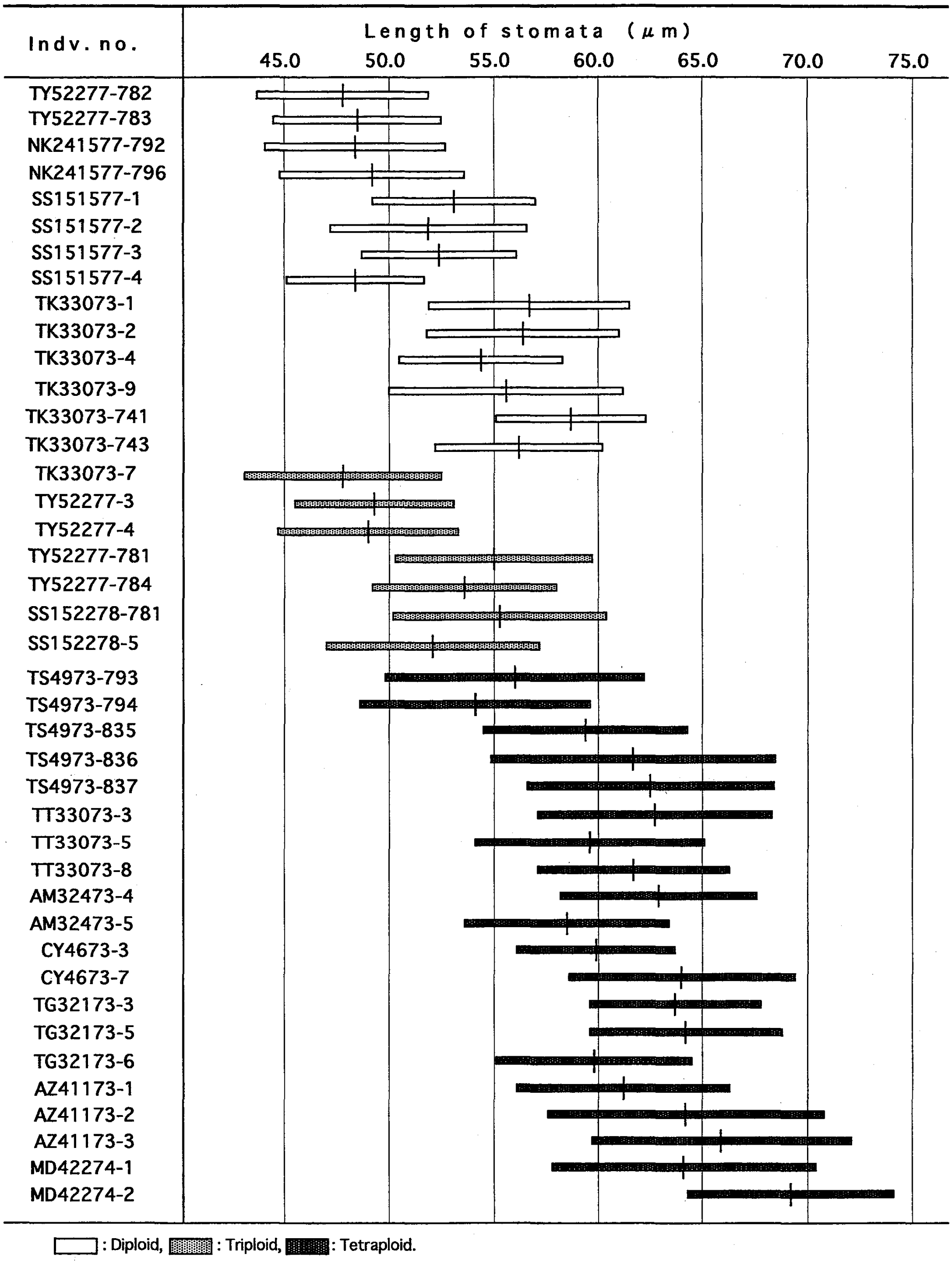


Fig. 1. The length of stomata from diploid, triploid, and tetraploid individuals. The mean value (vertical line) and standard deviation (horizontal bar) are presented. The stomatal size varies so widely within each ploidy level and so continuously among diploid, triploid, and tetraploid level that no clear boundary is drawn among them.

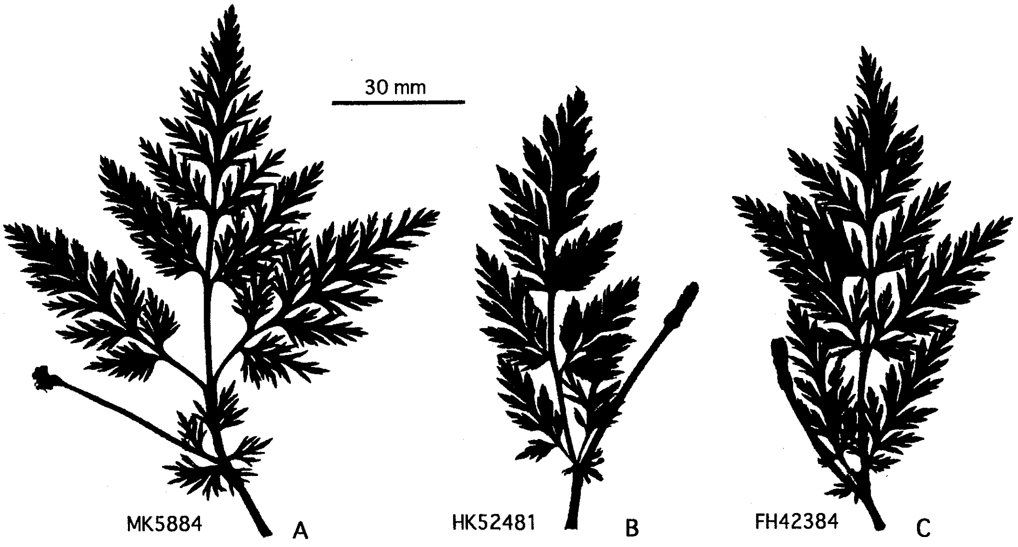
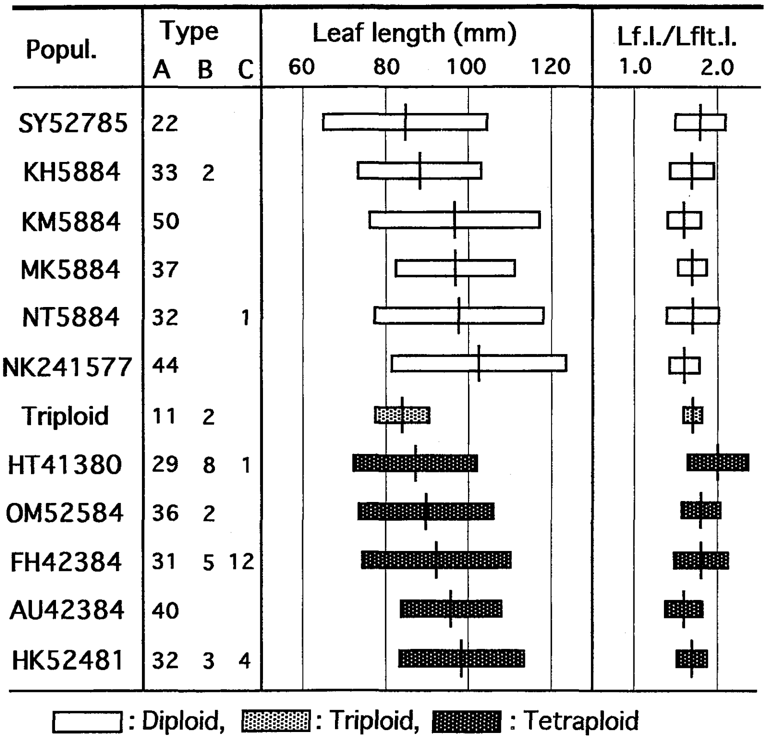


Fig. 2. Three types of bipinnate leaves. Type A: the first primary leaflets are longer than the second primary ones (MK5884), type B: the first and the second primary leaflets are similar in length (HK52481), type C: the second primary leaflets are longer than the first primary ones (FH42384). Type A is most common among diploid, triploid, and tetraploid plants.



Popul.	Type A	Type B	Type C
SY52785	22		
KH5884	33	2	
KM5884	50		
MK5884	37		
NT5884	32		1
NK241577	44		
Triploid	11	2	
HT41380	29	8	1
OM52584	36	2	
FH42384	31	5	12
AU42384	40		
HK52481	32	3	4

Fig. 3. The number of leaf types (A, B, C), the length of leaves (A) and the ratio of leaf length to primary leaflet length are summarized. The mean value (vertical line) and standard deviation (horizontal bar) are presented.

Table 2. Hairiness of leaves and pedicels of 15 individuals in diploid, triploid, and tetraploid *Adonis amurensis*

Ploidy level	Diploid	Triploid	Tetraploid
Adaxial side of leaves	sh	sh	sh
Abaxial side of leaves	sh	sh	+
Pedicels	sh	sh	++

sh: scarcely hairy, +: hairy.

plants, hairiness was a common character among them.

Other leaf characters such as degree of serration on secondary leaflets, density of hairiness on both surfaces of leaf, and petiole and petiolule length varied widely even among populations with the same chromosome number. Therefore, it was inappropriate to adopt these characters as a means of distinguishing ploidy level. The whole appearance of leaf also was little use to predict the ploidy level of *Adonis* plants (Fig. 4).

(3) *Anatomical Characters of Pedicels*

Pith cavity A reticular structure composed of parenchyma cells was observed in the pith cavity of pedicel in most tetraploid individuals, while not in diploiod individuals at the end of the fruiting season. In triploid individuals, only vestigial reticular structures were observed. At the peak of the blooming season, however, the reticular structure was observed more frequently in tetraploid, triploid, and diploid individuals (Figs. 5–6).

Number of vascular bundles Comparing mean values, the number of vascular bundles per pedicel was almost the same at the peak of blooming as at the end of fruiting seasons within the same ploidy level. Tetraploid plants showed the highest mean value (18.2 and 17.9), diploids the next (15.0 and 15.9) and triploids the lowest (13.0 and 13.4) (Fig. 7). But, when the standard deviation was taken into consideration, it became difficult to distinguish diploid, triploid, and tetraploid plants by making a count of vascular bundles.

Sclerenchyma cells In the present study a vascular bundle having less than five vessels was defined as a "small" vascular bundle, while the one with more than five vessels as a "large" one for convenience sake.

The presence of sclerenchyma cells was examined by using the pedicels collected at the peak of blooming and at the end of the fruiting season, respectively. The results are summarized in Fig. 8.

Sclerenchyma cells were always observed on the outer peripheral portion of large vascular bundles of pedicels that were sampled at the end of the fruiting season in both diploid and tetraploid plants. But in triploids, they were not observed in 51.2 % of samples (Figs. 8–9).

In the pedicels sampled at the peak of the blooming season they were not observed at all in triploid and tetraploid plants, while in diploids, they were observed in 67.2 % of samples (Fig. 8).

A similar tendency was recognized on the outer peripheral portion of "small" vascular bundles of pedicels collected at both seasons, though the frequency of their presence was lower than for the "large" vascular bundles (Fig. 8).

Discussion

Comparisons of stomatal size between diploid and tetraploid plants were made by Nishikawa and Ito (1978). They mentioned that both leaf size and stoma size were larger in 2n = 32 plants than in 2n = 16 plants. They presented two pictures of stomata but

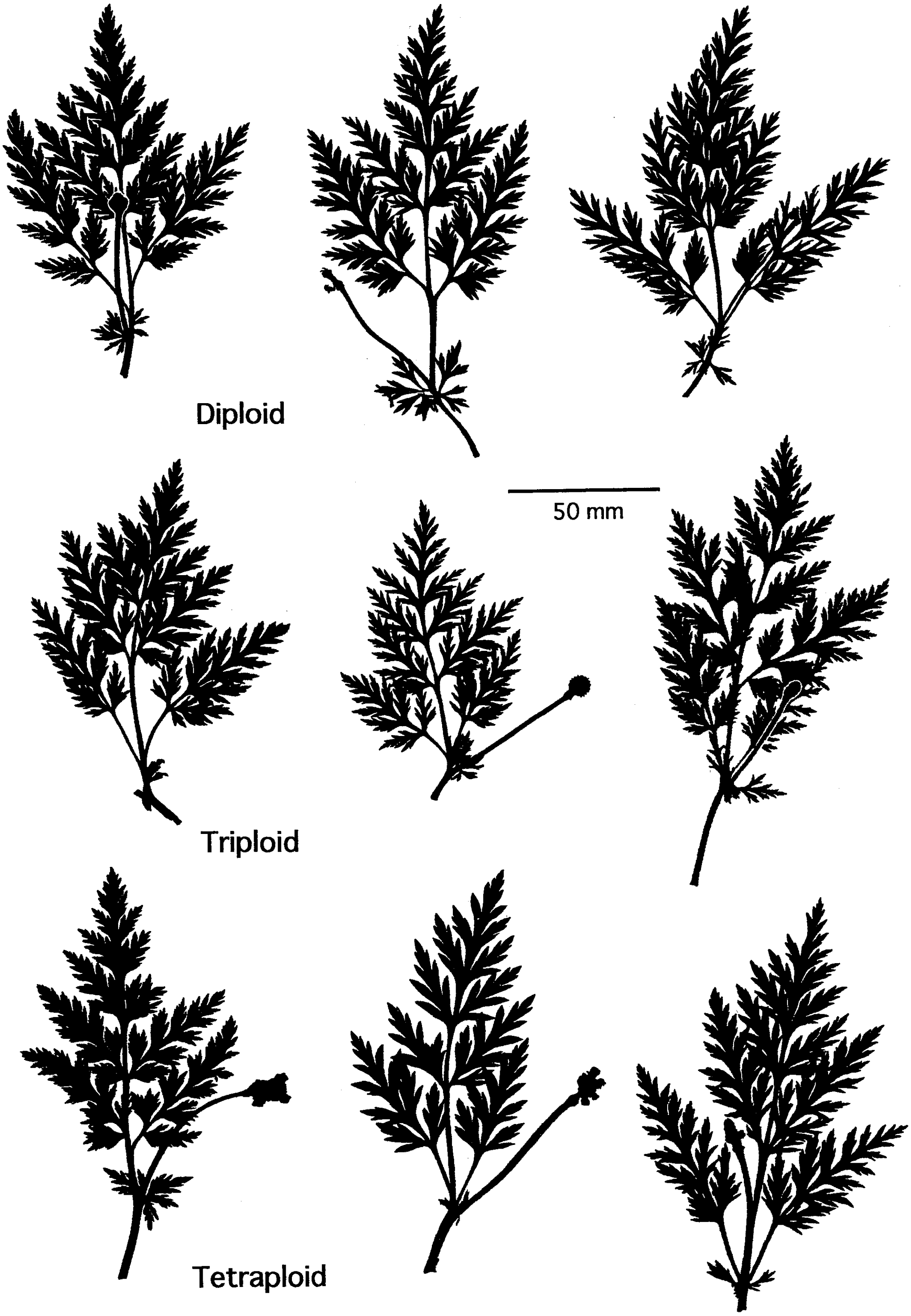


Fig. 4. Silhouettes of leaves (type A) from diploid, triploid, and tetraploid individuals. From left to right, diploid: KM5884, MK5884, NK241577, triploid: NK241577, NK141577, AS51584, and tetraploid: AU42384, OM52584, FH42384. Clear delimitation is impossible among diploid, triploid, and tetraploid individuals.

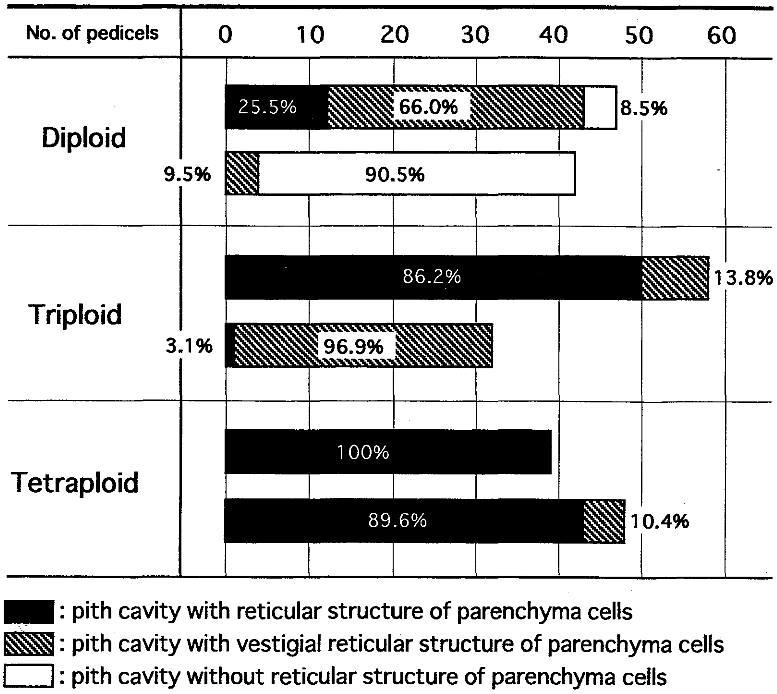


Fig. 5. Anatomical features of pith cavity of pedicels. Upper bar indicates pedicels collected at the peak of blooming, while the lower one indicates ones collected at the end of fruiting season. The reticular structure composed of parenchyma cells is present in common among diploid, triploid, and tetraploid individuals in early stages of the development of pedicels.

detailed data on which their statement was based were not included in the paper (Nishikawa and Ito 1978).

When the maximum and the minimum values are compared, there was a distinct difference in stomatal size between diploid and tetraploid plants (Fig. 1). But the size varied so widely within each ploidy level and so continuously among diploid, triploid, and tetraploid level that no clear boundary could be drawn among them (Fig. 1). Therefore, the present results do not support Nishikawa and Ito's statement (Nishikawa and Ito 1978).

Nishikawa and Ito reported that leaf size was larger in tetraploid plants than in diploid ones, showing a picture of two leaves from tetraploid and diploid plants (Nishikawa and Ito 1978). But they mentioned neither in the text nor in caption from what part of the plant and in what season the samples were collected. Therefore, it is inappropriate to compare the results I obtained with those they obtained.

In my previous papers I mentioned that the flowering period of tetraploid and diploid plants overlapped partly in natural populations around Morioka area (Suda and Herai 1991, Suda and Adachi 1991). At the mixed populations of diploid and tetraploid plants in Tamayama-mura and Iwate-machi we observed tetraploid plants with matured aggregate fruits and fully developed grayish-green leaves and diploid plants with flowers in full bloom and juvenile green leaves at the same time in the middle of April. In such mixed

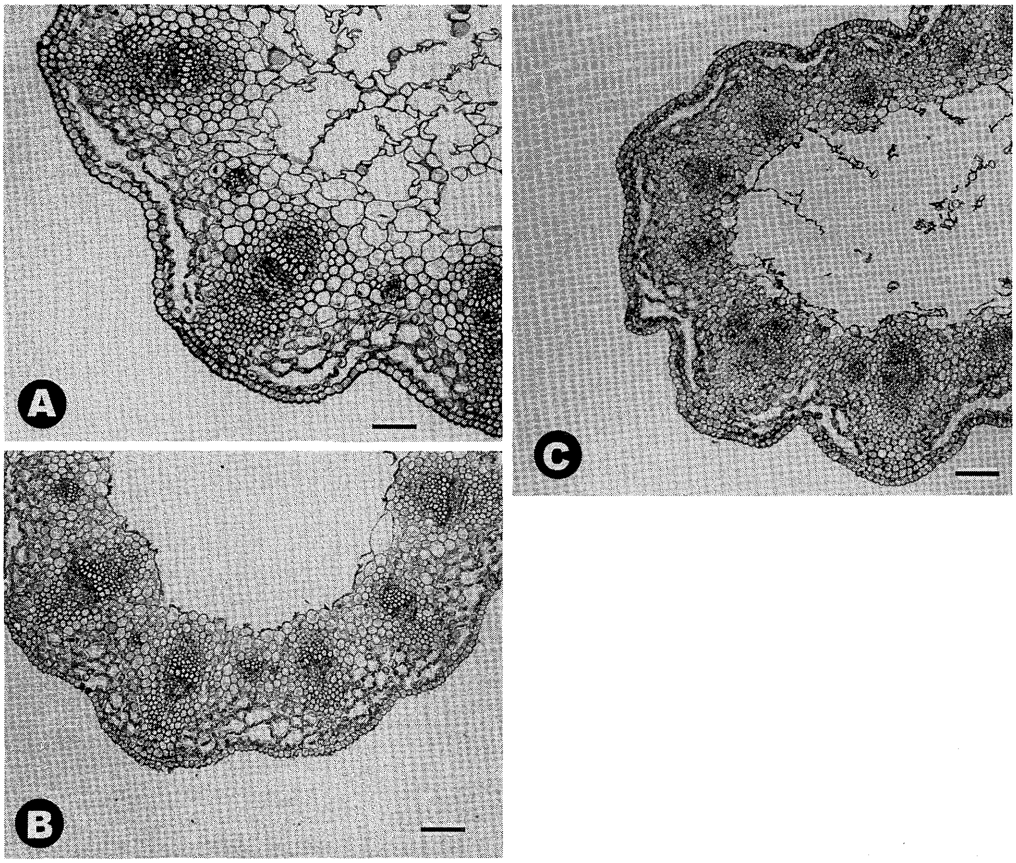


Fig. 6. Cross section of pedicels, showing three different types of pith cavity. A: Tetraploid (TS4973–5983) with reticular structure, B: Diploid (NK241577–2) without reticular structure, and C: Triploid (TK33073–7) with vestigial reticular structure. Samples were collected at the end of fruiting season. A scale bar inserted in each panel is equal to 100 μm.

populations it was easy to distinguish doploid and tetraploid by the size and color of leaves in this season.

Diploid and tetraploid plants were studied anatomically using cross sections of stems and cauline leaves by Nishikawa (1988). He mentioned that stems of the 2n = 16 Honshu specimens were hollow, but that the rest were solid, and that there was no difference between the 2n = 32 specimens in Hokkaido and Honshu. Further, he reported that the stems of the 2n = 16 Hokkaido specimens were more compactly solid than the 2n = 32 specimens (Nishikawa 1988).

Though the organs examined by Nishikawa and me were different, the results obtained were similar. The reticular structure composed of parenchyma cells was present in common among diploid, triploid, and tetraploid individuals in early stage of development of both stems and pedicels. Only a degree of compactness of the structure varied in parallel with the development of organs.

It is natural for the tetraploids with more sepals per flower to have more vascular bundles per pedicel than the diploids with fewer

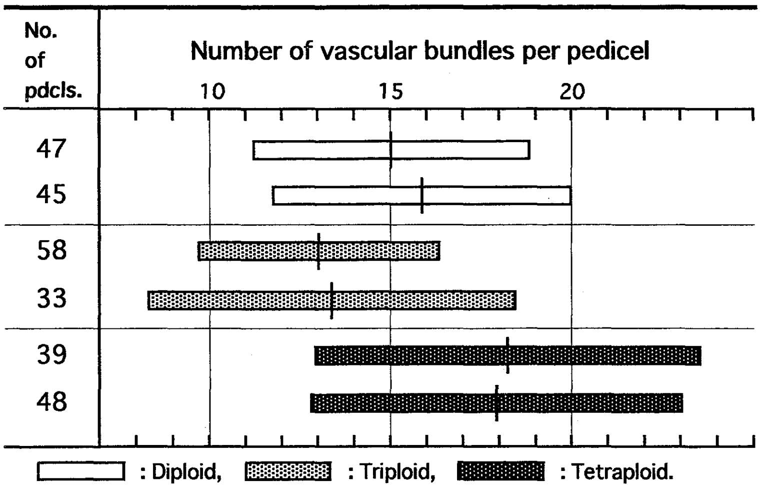


Fig. 7. Number of vascular bundles per pedicel. Upper bar indicates pedicels collected at the peak of blooming, while the lower bar indicates ones collected at the end of fruiting season. The mean value (vertical line) and standard deviation (horizontal bar) are presented. It is difficult to distinguish diploid, triploid, and tetraploid plants by making a count of vascular bundles.

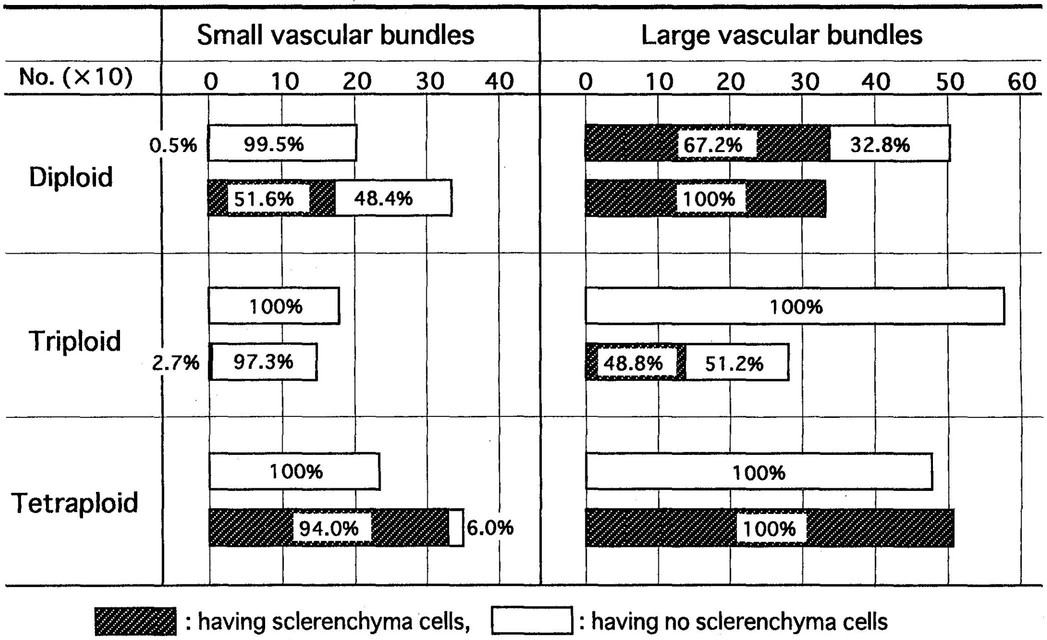


Fig. 8. Sclerenchyma cells formed on outer peripheral portions of vascular bundles. Upper bar indicates pedicels collected at the peak of blooming, while the lower one indicates ones collected at the end of fruiting season. Sclerenchyma cells develop in parallel with the development of pedicels.

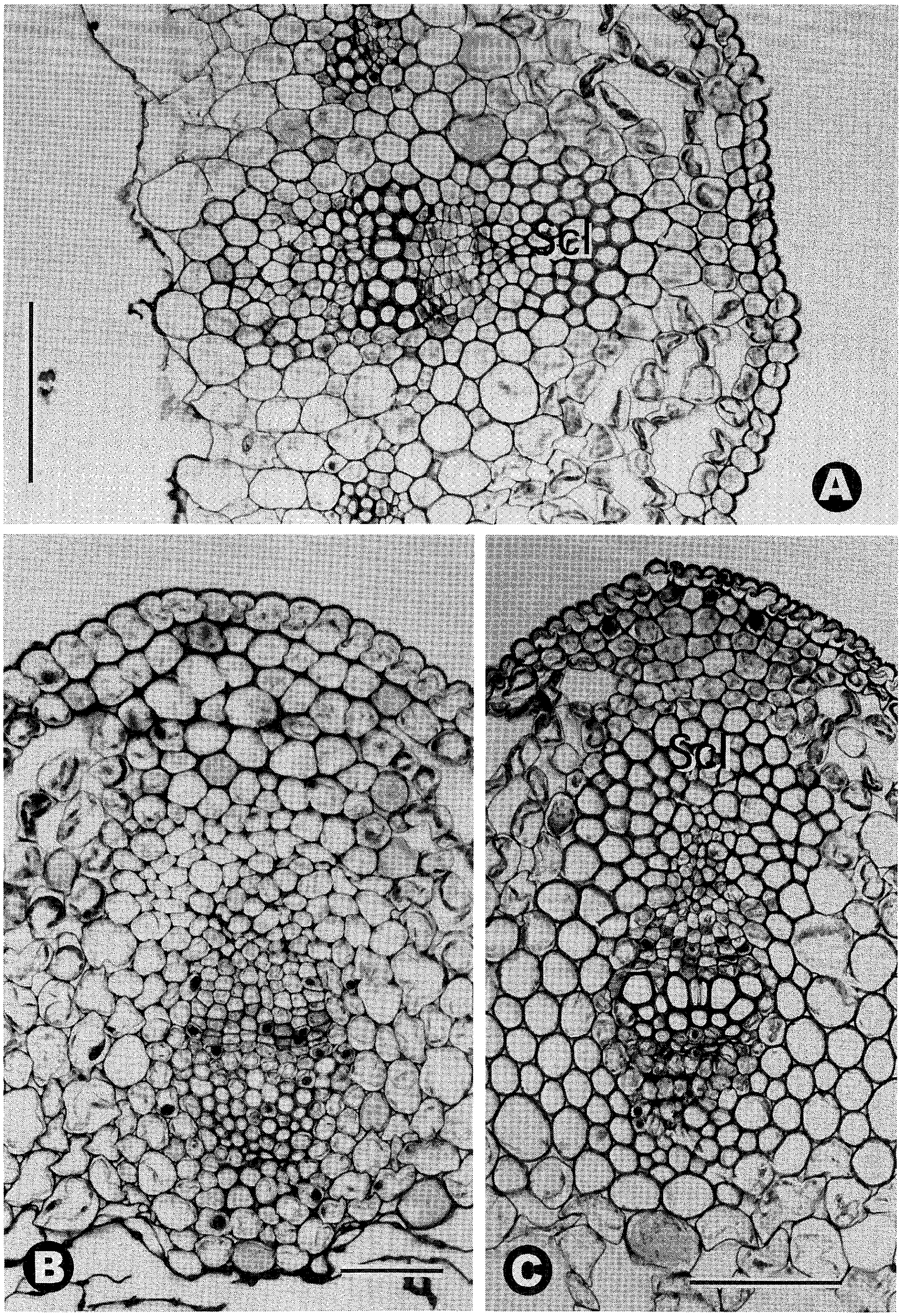


Fig. 9. Cross section of pedicel, showing sclerenchyma cells (Scl) on outer peripheral portions of vascular bundles. A: Diploid (NK241577–2), B: Triploid (TK33073–7), C: Tetraploid (AZ41173–2). Sclerenchyma cells are always observed in diploid and tetraploid plants at the end of fruiting season. A scale bar inserted in each panel is equal to 100 μm.

sepals per flower. However, the fact that the triploids, which have more sepals than diploids (Suda 1998), show fewer vascular bundles than diploids does not support the above speculation. Further examination will be necessary before a final conclusion is possible.

The appearance of sclerenchyma cells on the outer peripheral portion of vascular bundles has no relation to the polyploid variation of chromosomes. They appear in parallel with the development of pedicels.

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須田 裕：**邦産フクジュソウ属植物の分化（VI）葉の形質と小花柄**

葉と小花柄の形質を，邦産フクジュソウ属植物の二倍体，三倍体および四倍体の間で比較検討した．調査した形質は，結実期の小果柄の髄腔の構造を除けば，倍数性を区別するのに十分ではないことが判明した．先行研究の結果は再検討を要することが示唆される．

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